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Supplemental Trace Minerals (Zn, Cu, and Mn) as Sulfates or Hydroxy Trace Mineral Sources for Beef Heifers

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Supplemental Trace Minerals (Zn, Cu, and Mn) as Sulfates or Hydroxy Trace Mineral Sources for Beef Heifers

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ABSTRACT

Crossbred heifers (n = 286, 255 ± 4.5 kg initial BW, 295 ± 16.5 d of age) were used over a 2-yr period to determine the effects of mineral source on beef heifer development at 2 locations (n = 71 and n = 72, Fayetteville, blocks 1 and 4; n = 72 in each of 2 breeding groups, Batesville, blocks 2 and 3). Heifers were stratified based on initial BW, age, health, prior research projects, and sire, and then assigned to 6 groups of 12 heifers, that were assigned randomly to 1 of 2 trace mineral treatments. The 2 treatments were trace mineral supplementation (Cu [74 mg/d], Mn [294 mg/d], and Zn [221 mg/d]) as 1) sulfate or 2) hydroxychloride sources. Treatments were delivered through mineral and vitamin supplements provided free choice and formulated for a consumption rate of 113 g/d. Treatments began on d 0, and the breeding season began on d 112 and d 105 (blocks 1 and 4 respectively). After a synchronization period and a 10 d eligible period for artificial insemination, heifers were exposed to bulls for 50 d. At d 130 (block 2) and d 146 (block 3) heifers were exposed to bulls for 60 d. The trail concluded on d 224 (block 1), d 227 (block 4), d 252 (block 2), and d 268 (block 3). During the trial, BW at 28-d intervals, mineral disappearance, health records, and reproductive efficiency data were recorded. At the end of each trial, pregnancy was confirmed by the presence of Pregnancy-Specific Protein B concentrations in blood. No treatment differences ($P \geq 0.52$) were detected in BW or ADG. There was no significance for greater mineral disappearance between mineral sulfate and hydroxychloride treatments ($P = 0.46$) There were no differences in the percentage of heifers treated for bovine respiratory disease ($P = 0.77$) or foot rot occurrence ($P = 0.57$) between sulfate and hydroxychloride treatments. Trace mineral source did not affect overall pregnancy rates ($P = 0.85$). Therefore, supplementing either a sulfate or hydroxychloride source of Zn, Cu, and Mn to developing beef heifers resulted in similar performance.

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TABLE OF CONTENTS

Page

Chapter 1: Literature Review……1

Introduction……1

Cobalt (Co)……....2
Functions……...…2
Effects on Reproductive Performance……....2
Requirements……..3
Effects Due to Deficiency……...3
Other Mineral Interactions……..3
Bioavailability……4
Toxicity……...4

Copper (Cu)……4
Functions……...…4
Effects on Reproductive Performance……....4
Requirements……..5
Effects Due to Deficiency……...5
Other Mineral Interactions……..5
Bioavailability……6
Toxicity……...7

Iodine (I)……8
Functions……...…8
Effects on Reproductive Performance……....8
Requirements……..9
Effects Due to Deficiency……...9
Other Mineral Interactions……..9
Bioavailability……9
Toxicity……...9

Iron (Fe)……10
Functions…….....10
Effects on Reproductive Performance……...10
Requirements……11
Effects Due to Deficiency……..11
Other Mineral Interactions……11
Bioavailability……11
Toxicity……..12

Manganese (Mn)……12
Functions…….....12
Effects on Reproductive Performance……...12

Requirements……13
Effects Due to Deficiency……..13
Other Mineral Interactions……14
Bioavailability……14
Toxicity……..14

Selenium (Se)……15
Functions……..…15
Effects on Reproductive Performance……...15
Requirements……15
Effects Due to Deficiency……..16
Other Mineral Interactions……16
Bioavailability……16
Toxicity……..17

Zinc (Zn)……17
Functions……..... 17
Effects on Reproductive Performance……...18
Requirements……18
Effects Due to Deficiency……..18
Other Mineral Interactions……19
Bioavailability……19
Toxicity……..20

Conclusion……20

Chapter 2……....21

Introduction……21

Materials and Methods……...21
Experimental Treatments……...22
Heifer Growth and Health Measurements……23
Reproductive Data and Breeding Strategy……24
Calf Measurements……25
Laboratory Analyses……..25
Statistical Analyses……28

Chapter 3: Results and Discussion……29
Heifer Performance……...29
Mineral Disappearance……29
Heifer Health……30
Reproductive Performance……...31
Liver Mineral Concentration……32
Plasma Mineral Concentration……34
Implications……..35

Tables

Table 1. Nutrient composition of free choice mineral supplements for heifers……...36
Table 2. Nutrient composition of forage samples from pastures……..37
Table 3. Nutrient composition of hay supplements for heifers…….....38
Table 4. Nutrient composition of corn gluten supplements offered to heifers……..39
Table 5. Mineral composition of water…….40
Table 6. Comparison of heifer health…….....41
Table 7. Comparison of reproductive performance……...42

Figures

Figure 1. Body weight gains……..……...43
Figure 2. ADG for treatments……..……44
Figure 3. Comparison of HCS……....……...45
Figure 4. Mineral Disappearance……...……...…46
Figure 5. Liver Copper Mineral Concentrations……..…….47
Figure 6. Liver Zinc Mineral Concentrations…….....48
Figure 7. Plasma Copper Concentrations…….....49
Figure 8. Plasma Zinc Concentrations……..…50

References……51

Appendix……...58
IACUC Letter……58

Chapter 1

Review of Literature

With advancements in disciplines, such as animal health, pasture management, and reproductive performance, livestock agriculture, including beef production, has become more of a performance-based system. In order for these animals to produce to their greatest genetic potential, it is of major importance to make sure their greater nutritional demands are met. Greater performing and productive cattle result in greater nutritional demands for energy, protein, vitamins, and minerals. According to Greene (2000), beef cattle can derive a large part of their nutritional demands from forages. However, forage alone does not provide a complete feed for beef cattle nutritional requirements (Greene, 1997). For any animal to maintain long-term production, mineral supplementation is necessary (Greene, 2000). Ammerman and Goodrich (1983) stated that the importance of mineral elements on the health and well-being of animals and humans has been recognized for centuries, although individual elements involved were unknown.

Mineral requirements of beef cattle include both macro- or major minerals and micro- or trace minerals. Macrominerals are normally required in larger amounts by beef cattle, thus receiving the name macro- or major. Beef cattle requirements for macro minerals are determined based on the stage of life and level of production (NASEM, 2016). Micro- or trace minerals are normally required and supplied in much smaller quantities. Trace mineral requirements tend to remain very similar for all stages of life or production, with one exception, Mn (NASEM, 2016).

This literature review will focus on trace mineral elements and how they affect bodily functions and reproductive performance. In addition, this literature review will look at the

requirements, the effect of trace mineral deficiency, how the mineral interacts with other minerals, bioavailability of the mineral, and toxicities of these required trace minerals.

COBALT (Co)

Functions

The requirement for dietary cobalt was not known, until investigations into the underlying cause of 'coast disease' in sheep (Lines, 1935; Marston, 1935) and 'wasting disease' in cattle in Australia and New Zealand (Underwood and Filmer, 1935; Suttle, 2010). In the late 1940s, researchers were able to prove that cobalt is a major factor in the structure of vitamin B_{12}. Just as calcium is needed in bone development and growth, Co is needed for vitamin B_{12} synthesis. Vitamin B_{12} compounds are essential cofactors for a variety of enzymes involved in propionate metabolism and synthesis of methane, acetate, and methionine in various species (Hussein et al., 1994). Cobalt supplementation improves digestion of high fiber feedstuffs in the rumen by enhancing bacterial activity, and increases rumen volatile fatty acid concentrations (Hussein et al., 1994).

Effects on Reproductive Performance

Field experience suggests that Co deficiency impairs breeding performance in both cattle and sheep. In Co deficient locations in Africa and South America, cattle have a 24-mo calving interval versus the typical 14-mo period in non-deficient locations (Hidiroglou, 1979). Hidiroglou (1979) also suggested that the most common manifestation of Co deficiency, related to reproduction, is a reduction in conception rate. Cows supplemented with Co have a stronger manifestation of estrus, lower occurrence of irregular estrus cycles and greater conception rates (Hidiroglou, 1979).

Requirements

Cobalt concentrations can vary between plant species and with soil conditions (Minson, 1990). Also, there is typically less Co supplementation needed on legume pastures compared to grass pastures, as long as the soil is not deficient (Looney et al., 1976). The data on Co in grains and other concentrates are not well established, partly because of the difficulty in measuring low concentrations in silica-rich samples (Suttle, 2010). Because of this, feedstuffs such as cereal grains, are a poor cobalt source (Suttle, 2010), the requirement for Co is 0.10 mg/kg DM for cattle in all stages of production (NASEM, 2016).

Effects Due to Deficiency

Cobalt deficiency in ruminants results in vitamin B_{12} deficiency due to an inability of the rumen microorganisms to synthesize sufficient vitamin B_{12} from their cobalt supply (Suttle, 2010). A diet that is deficient in Co, can lead to anemia, reduced appetite, decreased growth, reduced body weight, and can ultimately lead to death. With a vitamin B_{12} deficiency, methylmalonic acid accumulates, and in turn causes elevated concentrations of methylmalonic acid in plasma and urine (Kincaid, 2000). Cobalt deficiency is most critical in certain areas of the United States, including parts of New England and parts of the Atlantic Coastal Plains. The remainder of the United States, especially the Midwest Region, yield forages that are moderately deficient in Co.

Other Mineral Interactions

Animals provided with adequate levels of Co are less susceptible to selenium (Se) toxicosis (Gardiner, 1966).

Bioavailability

In the beef cattle industry, the major sources of Co supplementation are cobaltous carbonate, cobaltous sulfate, and cobalt glucoheptonate (Ammerman et al., 1995), with cobaltous sulfate being the standard. The two most non-bioavailable forms of cobalt are cobaltic-cobaltous oxide and cobaltous oxide (Ammerman et al., 1995).

Toxicity

The maximum tolerable level for Co is 15 mg/kg DM, but since vitamin B12 is not stored in the body, Co toxicity is unlikely to occur in beef cattle (NASEM, 2016).

COPPER (Cu)

Functions

According to Suttle (2010), Cu is present in and essential for the activity of numerous enzymes, cofactors, and reactive proteins in the body. Copper also has many essential functions in tissues that make up the body. Therefore, a dietary deficiency can be implicated as a cause of infertility, anemia, or suppressed immune functions (Underwood and Suttle, 1999).

Effects on Reproductive Performance

Copper deficiency in the body can have detrimental effects on overall reproductive performance. According to Hidiroglou (1979), Cu deficiency can lead to prenatal mortality, particularly early embryonic loss; and one of the earliest symptoms of Cu deficiency in cows is infertility. Infertility and abortions have also been reported in experimental Cu deprivation of ewes (Howell and Hall, 1970). Copper concentrations in plasma tend to decrease in ewes during gestation and rise after parturition, but in cows, Cu concentrations have been found to be greater levels around 5 months of gestation (Hidiroglou, 1979). During the gestation period of a cow, the

majority of Cu is directed into the fetus, instead of storing it in the body. Even when pregnant cows are fed Cu deficient diets, their calves bled at 7 d of age had serum Cu concentrations that were not different from calves born to cows fed to meet their Cu requirement (Gengelbach et al., 1994).

Requirements

The Cu requirement for beef cattle is 10 mg/kg DM (NASEM, 2016). Copper requirements can vary among the numerous breeds of cattle. Several studies have shown that when fed the same level of dietary Cu, Continental breeds of cattle had lower plasma Cu concentrations compared to British breeds of cattle (Ward et al., 1995). A study completed by Littledike et al. (1995) found that Limousin cattle also have greater Cu concentrations compared to most other breeds with the exception of Angus. According to Underwood (1977), normal plasma Cu concentrations are considered to be approximately 0.8 to 1.2 mg/L, while values less than 0.6 mg/L are considered deficient.

Effects Due to Deficiency

Copper levels vary in forages, with non-legume forages tending to be lower than 5 mg/kg, while legumes tend to have at least 5 mg Cu/kg DM (Engle et al., 1964). When a Cu deficiency is present, many different signs can occur including ataxia, abnormalities in the crimp of wool, infertility problems, anemia, and fragile bones. The most common visual sign is hair depigmentation.

Other Mineral Interactions

Excess molybdenum (Mo), iron (Fe), and zinc (Zn) can inhibit Cu utilization and storage by forming insoluble Cu complexes in the digestive tract, bloodstream, and tissues of ruminants (Dick et al., 1975). It is not uncommon to find Fe levels in forage well over 2 times the

requirement for beef cattle, and excess Fe has a detrimental effect on the bioavailability of Cu (Greene, 2000). Sulfur (S) can also interfere with Cu uptake with a common source being molasses-based supplements having high S concentrations, which in turn reduces Cu availability overall. This interference can cause the formation of ruminal thiomolybdates (Mason, 1990). Arthington et al. (2003) showed an increase in dietary S from 0.1 to 0.4% may result in a 50% increase in the overall dietary Cu requirement. Wellington et al. (1998) stated that caution must be exercised when increasing Zn supplementation without increasing Cu supplementation, do to Zn and Cu interacting and decreasing the amount of Cu being available to the animal.

Bioavailability

Copper sources can fall into inorganic and organic mineral sources. Organic sources may be more bioavailable by chelating or complexing them to amino acids, peptides, proteins to attempt to improve availability. Arthington and Pate (2002) reported that organic Cu forms, such as amino acid complexes, may be more bioavailable than $CuSO_4$ to cattle grazing forages high in sulfur. Kirchgessner and Grassman (1970) also speculated that the addition of amino acids were needed for brush border uptake, in the small intestine, of Cu, which would increase Cu availability and improve performance. The authors' speculation is supported by later findings indicating that stressed calves fed Cu-lysine had a 53% greater apparent Cu absorption and retention compared to those calves fed $CuSO_4$ (Nockels et al., 1993). A study using organic sources of Cu, Mn, Zn, and Co (4-Plex, Zinpro Corp., Eden Prairie, MN) that found that cows supplemented with the organic form of mineral had a shorter postpartum interval to breeding compared to cows supplemented with inorganic sulfate forms, as well as unsupplemented controls (Swensen et al., 1998).

However, there also has been contradictory research that is comparable to the above studies. Contradictory to the Swensen et al. study (1998), a similar trial using the same amino acid complexed Cu mineral sources found that supplementing first parity cows reduced reproductive performance compared to the unsupplemented first parity cows (Olson et al., 1999). Furthermore, in a study where inorganic Cu sulfate ($CuSO_4$) and organic Cu-lysine complex were compared, they were similar in bioavailability for Cu deficient calves (Kegley and Spear, 1994). When both organic and inorganic mineral sources were fed to heifers receiving adequate Cu in molasses supplements, there were no differences found between organic amino acid complexed Cu and inorganic Cu sulfate sources for liver Cu concentrations (Arthington et al., 2003).

With research supporting and contradicting the bioavailability of Cu in organic and inorganic forms, there are many inconsistencies. The inconsistency of these trials could be due to feeding available Cu in excess of requirements (Muehlenbein et al., 2001).

Toxicity

The maximum tolerable level of Cu for any stage of life, in a beef cow, is 40 mg/kg (NASEM, 2016). There are 3 stages to Cu toxicity, which are the gradual accumulation of Cu in the liver, with no clinical signs and blood Cu levels appearing to be normal, then the blood Cu increases to about 2 times the normal level, and finally, the animal goes into a hemolytic crisis with signs of dullness, anorexia, dehydration, acute thirst, and death occurring within 2 to 4 d after onset (Suttle, 2010). Copper toxicity usually occurs due to the overconsumption of plants or feeds that have a greater level of Cu, excessive consumption of mineral mixes that have greater Cu concentrations, improper use of Cu drenches, and consumption of agricultural Cu compounds, such as insecticides and fungicides (Suttle, 2010). Copper toxicity is easily

prevented by increasing the level of Mo and S in the animal's diet, administration of thiomolybdate, or the administration of D-penicillamine (Suttle, 2010).

IODINE (I)

Functions

Ancient Greeks were said to have fed burnt sea sponges to people to reduce the size of an enlarged thyroid, and it was later found that these sponges contained iodine (Ammerman and Goodrich, 1983). Even though the Greeks did not know at the time, they discovered that iodine was one of the first important trace minerals needed for bodily functions. Iodine is needed in the formation of thyroxine and normal ovarian function (Suttle, 2010). Suttle (2010) also mentions that long-term intake of moderate to high levels of iodine, will reduce iodine uptake by the thyroid gland. The involvement in thyroid function and homeostasis of the gland and hormones, thyroxine and triiodothyronine, is understood fairly well (Hidiroglou, 1979).

Effects on Reproductive Performance

Hidiroglou (1979) concluded that an iodine deficiency during gestation will result in impaired fetal thyroid functions, and abortions are common among iodine deficient cows. Normal development of the heifer reproductive system is dependent on the animal's thyroid status. It also has been shown that thyroidectomized heifers ceased to exhibit estrus at regular intervals (Speilman et al., 1945). A deficiency of iodine can also lead to irregular estrus and retained placentas (Hidiroglou, 1979). Suttle (2010) also mentions that when iodine is properly supplemented, it resulted in greater conception rates and lower incidence of retained placentas, compared with cows that were not supplemented with iodine.

Requirements

The requirement for iodine in all stages of production and growth in beef cattle is 0.50 mg/kg of DM (NASEM, 2016).

Effects Due to Deficiency

According to Greene (2000), in the United States, a widespread iodine deficiency does not exist, but deficiencies do occur in specific regions of the country. The most frequently observed signs of iodine deficiency among domestic animals are the presence of goiter and the birth of weak or dead offspring (Ammerman and Goodrich, 1983).

Other Mineral Interactions

There are no major interactions of iodine with other minerals that will cause interferences.

Bioavailability

In the beef cattle industry, the major sources of iodine supplementation are potassium iodide, sodium iodide, and calcium iodate. According to Ammerman et al. (1995), calcium iodate is a form commonly added to cattle diets and has slightly lower bioavailability compared to other forms of supplemental iodine. Iodine is often supplemented in the more bioavailable organic form of ethylenediaminedihydriodide that in therapeutic doses can prevent potential problems that occurred in this country prior to iodine being added to salt (Greene, 2000).

Toxicity

The maximum tolerance level of iodine listed for cattle is 50 mg/kg of DM (NASEM, 2016).

IRON (Fe)

Functions

Iron is by far the most abundant trace mineral in the body and its value as a dietary constituent has been appreciated for over 2,000 years (Suttle, 2010). Iron was found to be present in the blood in 1832, but ancient Greeks knew that if a person was suffering from anemia, drinking rusty water would cure the condition (Ammerman and Goodrich, 1983). Approximately 60% of Fe in the body is present as hemoglobin (Hb) in the bloodstream (Suttle, 2010). Iron is mostly present and involved in cellular respiration. It is intricately involved in oxygen transport and storage in the tissues because its presence in hemoglobin and myoglobin facilitates oxygen-binding capacity (Suttle, 2010). In addition, Fe-containing cytochrome enzymes facilitate oxygen utilization at the cellular level (Ammerman and Goodrich, 1983).

Effects on Reproductive Performance

Due to the high amounts of Fe in the soil, not much research has been completed on its effect on reproductive performance of grazing beef cattle, since the animal receives more than enough Fe from the soil. The most common incidence of Fe deficiency in reproductively efficient cattle is due to disease or external or internal parasites. This has drawn researchers to look at the interaction of Fe with reproductive performance. According to Hidiroglou (1979), a study showed that Fe, Mn, Cu, and Zn concentrations were significantly greater in serum of regular breeders compared to repeat breeders, and Fe and Zn concentrations were greater in cows who conceived with fewer inseminations. Also, Ferrell et al. (1982) found that providing adequate Fe in feedstuffs or by supplementation is essential for gestating beef cows since the Fe content in bovine fetuses increases from 43 to 62 mg/kg from d 100 to 280 of gestation.

Requirements

Iron is required by all classes of cattle at 50 mg/kg of DM (NASEM, 2016).

Effects Due to Deficiency

Iron deficiency does not occur often in grazing beef cattle in the United States (Greene, 2000). The most common reasons for Fe deficiency to occur is disease, injury, and external or internal parasites. With this being said, there has never been an Fe deficiency occurrence officially reported in grazing cattle. This is due to the abundance of Fe found in the soil of forages and all feedstuffs. This is supported by Hidiroglou (1979), with Fe being abundant in all feeds, deficiency in adult ruminants seems improbable. If deficiency was to occur, a reduction in circulating hemoglobin is one of the earliest signs of dietary Fe deficiency (Ammerman and Goodrich, 1983). Anemia is another sign of Fe deficiency, but this occurrence is more prominent in swine than cattle, because of forage intake. As previously stated Fe deficiency is not typically a problem with the exception of young ruminants such as milk fed calves (Kincaid, 2000).

Other Mineral Interactions

The most common interaction that Fe has with any other mineral is Cu. Copper is needed for Fe metabolism and pyridoxine deficiency decreases absorption (Suttle, 2010). This is supported by Greene (2000), showing that elevated levels of dietary Fe also act to exacerbate a Cu deficiency.

Bioavailability

Ferric citrate and ferric EDTA are more bioavailable to cattle than ferric phytate and ferrous carbonate (Henry and Miller, 1995). Iron salts such as iron oxide (FeO) are often added to free choice mineral supplements at 1% or higher as coloring agents to satisfy the perception by

producers that all good minerals have to be red in color, although it is not very absorbable and can have negative effects on Cu status in cattle (Greene, 2000).

Toxicity

The maximum tolerable level for all stages of cattle is 1,000 mg/kg of DM (NASEM, 2016). With such a high maximum tolerable level, Fe toxicity in ruminants is seldom a problem. (Ammerman and Goodrich, 1983).

MANGANESE (Mn)

Functions

Manganese is an important trace mineral in biological systems, acting as an enzyme component and activator (Leach and Harris, 1997). There has only been limited research completed on Mn, making it one of the least understood minerals (Hansen et al., 2006). According to Suttle (2010), Mn is needed for many functions in the body, such as amino acid metabolism, cholesterol metabolism, and activation of enzyme systems involved in oxidative phosphorylation, bone formation, growth, and reproduction. Manganese is also needed as a precursor in the formation of cholesterol, which is needed for reproductive purposes. Cholesterol is needed for the production of steroid hormones, and it is possible that decreased cholesterol in Mn deficient cattle could result in a delay in onset of estrus (Hansen et al., 2006). Also, the production of Mn superoxide dismutase is important in regulating luteal function and maintaining steroidogenesis (Olson et al., 1999).

Effects on Reproductive Performance

According to Hidiroglou (1979), Mn is necessary for normal fertility in beef cattle, and feeding diets low in Mn depresses conception rates in beef cattle. Depressed or delayed estrus

and poor conception rates have been associated with experimental Mn deprivation in cows, goats, and ewes (Underwood and Suttle, 1999). In a study completed by Hansen et al. (2006), heifers supplemented with above normal levels of Mn had greater offspring birth weights as well as fewer congenital abnormalities such as dwarfism in calves. The above statements are also supported by the Hidiroglou (1979) review that stated cattle deficient in Mn have been observed to have suppressed estrus, reduced conception rates, increased abortion rates and lighter calf birth weights. In bulls, Mn deficiency can lead to problems, such as testicular degeneration, while in cows or heifers it can lead to defective ovulation or abortions (Suttle, 2010).

Requirements

Few grazing areas in the U.S. and the world have been identified as deficient in Mn, so grains and forages normally contain adequate levels of Mn (Ammerman and Goodrich, 1983). Requirements for Mn are 20 mg/kg DM for growing cattle, and 40 mg/kg DM for gestating and lactating females (NASEM, 2016).

Effects Due to Deficiency

It becomes difficult to identify when a Mn deficiency is present, due to the symptoms being fairly similar to other mineral deficiencies. According to Greene (2000), identification of Mn deficiencies is difficult due to the inconspicuousness of symptoms. In sheep, lack of Mn causes difficulty in standing and joint pain, with poor locomotion and balance (Lassiter and Morton, 1968). When heifers are deprived of Mn, their calves have lighter birth weights and show disproportionate dwarfism, superior brachygnathism (‘undershot’ lower mandible), swollen joints and an unsteady gait (Hansen et al., 2006). Manganese is often supplemented just to prevent any deficiencies that may exist although forages normally contain adequate levels of Mn (Greene, 2000).

Other Mineral Interactions

Manganese absorption can be affected when greater levels of Ca and/or P are added to diet, due to the probability of these elements forming an insoluble complex. When Fe and Co have greater concentrations, they compete for and interfere with absorption sites in the small intestine (Suttle, 2010). During Fe and Mg deficiencies, there tends to be large increases in Mn absorption. Phytate and fiber also interfere with Mn absorption (Suttle, 2010). The addition of Zn can have a negative effect on in vitro DM digestibility of forages, but the addition of Mn seemed to counteract this effect (Arelovich et al., 2000).

Bioavailability

Manganous oxide (MnO) and Mn sulfate ($MnS0_4$) are used most frequently for supplemental sources of Mn (Ammerman and Goodrich, 1983). Manganese methionine is more bioavailable to sheep and would be assumed to be similar in cattle, as well as $MnS0_4$ compared to the oxide forms (Henry, 1995).

Toxicity

The maximum tolerable intake level for beef cattle is 1,000 mg/kg DM (NASEM, 2016). Manganese toxicity is unlikely to occur, but situations in where feeds were relatively rich in Mn, cattle had more abortions and cystic ovaries (Hidiroglou, 1979). Continuous exposure to levels over 200 mg/kg DM or higher in grazing forage have resulted in reproduction abnormalities in dairy cattle (Fonseca and Davis, 1969).

SELENIUM (Se)

Functions

Selenium is a component of many different enzymes in an individual's or animal's body. Such enzymes include iodothyronine 5'-deiodinase, thioredoxin reductase, selenophosphate synthetase 2, and most importantly glutathione peroxidase. According to Suttle (2010), glutathione peroxidase is used in the reduction of peroxides that arise from lipid tissue oxidation. The enzyme is involved in the destruction of hydrogen peroxide and lipid peroxides, and thus protects cell membranes from damage (Ammerman and Goodrich, 1983).

Effects on Reproductive Performance

Selenium deficiency greatly impacts reproductive performance, causing embryonic and fetal losses. In several cases of Se deficiency, supplementation of Se to cattle prevented certain reproductive problems (Hidiroglou, 1979), such as retained placenta, reduced cyclisity, and reduction in male fertility. Fertility seems less vulnerable to Se deprivation in grazing cattle (Wichtel et al., 1998), but has been improved by Se supplementation in housed heifers (MacPherson et al., 1987). In cattle, delayed expulsion of the afterbirth can be a Se-responsive condition (Underwood and Suttle, 1999). Selenium supplementation has reduced the incidence of endometritis and cystic ovaries (Harrison et al., 1984) and indirect improvements in fertility results from decreases in these and other reproductive disorders, including retained placenta (Suttle, 2010). Male fertility may be adversely affected and reduced viability of sperm has been reported in Se-deprived bulls (Slaweta et al., 1988).

Requirements

Selenium is required by all cattle at 0.10 mg/kg of DM (NASEM, 2016). Cows in late pregnancy require 3 to 5 mg of Se/d to ensure adequate Se levels in muscle tissues of newborns

(Abdelrahman and Kincaid, 1995). Relatively large amounts of Se are transferred to the fetus from the dam during the last trimester of pregnancy (Kincaid, 2000). Currently the FDA allows up to 0.3 mg/kg dietary Se to be supplemented (Greene, 2000).

Effects Due to Deficiency

Selenium deficiency can cause detrimental effects, such as loss of hair, horns, and hooves. The most significant sign of Se deficiency is called nutritional muscular dystrophy or white muscle disease and is a major sign of deficiency in ruminants (Ammerman and Goodrich, 1983). Although deficiencies of Se severe enough to produce clinical signs of white muscle disease are scarce in grazing cattle, subclinical deficiencies do occur and often affect production, efficiency and health (Greene, 2000). It has been recognized by many researchers and scientists that Se deficiency is a much more serious problem in ruminants than the toxicity of the element.

Other Mineral Interferences

There are no other major trace mineral interferences known to occur with Se.

Bioavailability

Selenium concentrations in forages are highly dependent on soil Se levels (Greene, 2000). It is commonly known that Se concentrations in legume forages are greater than in normal warm season grasses, such as Dallas and Bahia grasses. The efficiency of maternal transfer of Se to the fetus is affected by the chemical form of Se in the diet (Suttle, 2010). Selenomethionine is a much more bioavailable form of Se to ruminants than selenite (Ehlig et al., 1967). Additionally, more of the Se from selenomethionine is transferred to the fetus and milk (Kincaid and Rock, 1999).

Toxicity

Selenium toxicity can appear in two forms, chronic and acute toxicity. Chronic toxicity can cause blind staggers, if fed between 10 and 20 mg/kg DM, or alkali disease if fed between 5 and 10 mg/kg DM. Chronic toxicity signs include lameness, sloughing of hooves, loss of hair, and damage to liver and brain. Acute toxicity and sudden death occur in anything fed containing above 20 mg/kg Se. According to NASEM (2016), the maximum tolerable level for any stage of life in beef cattle is 5 mg/kg DM.

ZINC (Zn)

Functions

The first unequivocal evidence that Zn is necessary for growth and health was obtained in laboratory animals (Todd et al., 1934). Continuing biochemical studies reveal there are over 300 Zn-dependent enzymes of diverse structure and function (Vallee and Falchuk, 1993) and a far greater number of functional Zn proteins (Coleman, 2002), making it difficult to identify the rate limiting factor in Zn-responsive disorders (Suttle, 2010). Zinc has been recognized for several decades as indispensable for normal growth and health in animals (NASEM, 2016). Zinc is required for maintenance of skin integrity, stabilization of membranes, and activation of the cell-mediated immune system (Miller and Madsen, 1992). Greater feed efficiency and average daily gain were observed when heifers were fed 25 mg Zn/kg DM in addition to the 24 mg Zn/kg DM in a corn silage ration for the first 56 d of a study, but later in the feeding period no advantage was seen (Spears, 1989).

Effects on Reproductive Performance

Reproductive failure in the female and impaired spermatogenesis in the male are manifestations of Zn deficiency (Hidiroglou, 1979). Although naturally occurring clinical Zn deficiency is rare in livestock, there are numerous reports of improvements in the reproductive performance of animals given supplemental Zn (Hidiroglou, 1979). In pregnant females, severe Zn deficiency results in embryo death, small fetuses, and malformations (Masters et al., 1986). Hypogonadism occurs in Zn-deprived bull calves (Pitts et al., 1966), kids (Miller et al., 1964), and ram lambs (Underwood and Somers, 1969). Cows supplemented with Zn had a 23% greater conception rate compared to the control animals (Hidiroglou, 1979).

Requirements

The Zn requirement for beef cattle is 30 mg/kg DM (NASEM, 2016). Zinc requirements are not well defined and little is known regarding factors that may influence Zn requirements of cattle (NASEM, 2016). Optimal Zn requirements are rarely met by forages for cattle grazing pastures and Zn is the most deficient trace mineral in legume forages (Greene, 2000).

Effects Due to Deficiency

A Zn deficiency will result in poor hair development, and a deficiency is consistently associated with increased morbidity and mortality rates (Kincaid et al., 1997). Zinc deficiency can also cause a loss of appetite, or anorexia. Bowing of the hind limbs, stiffness of the joints and swelling of the hocks occur in calves deprived of Zn (Miller and Miller, 1962). Zinc deprivations can also cause the thickening, hardening and fissuring of the skin, or parakeratosis (Suttle, 2010). Parakeratosis sites vary between species, but common sites include the muzzle, neck, ears, scrotum and back of the hind limbs in calves (Miller et al., 1965a); the hind limbs and teats in the dairy cow (Schwarz and Kirchgessner, 1975); and around the eyes, above the hoof

and on the scrotum in lambs (Ott et al., 1964). Other studies have found that marginal Zn deficiency will decrease feed efficiency but not intake in heifer calves (Engle et al., 1995).

Other Mineral Interferences

It has been shown, that steers supplemented with 1,000 mg Fe/kg DM had reduced liver Zn concentrations (Standish et al., 1971) showing that high Fe intake interferes with Zn bioavailability due to coordinate covalent bonding between the metal and the organic functional groups (Mullis et al., 2003). High Ca levels and phytate bind with Zn making it insoluble and unavailable to ruminants. Excess Zn can also interfere with Cu metabolism and lead to anemia. Several authors concluded that supplementing Cu along with Zn could possibly have increased both absorption and retention of Zn (Hatfield et al., 2001).

Bioavailability

The common forms of Zn used to supplement animal rations are the oxide (ZnO) and feed-grade sulfate ($ZnSO_4$; Suttle, 2010). It is hypothesized that organic or chelated forms of Zn may interact less than inorganic sources of Zn with Fe and would be more bioavailable in the presence of high dietary Fe (Mullis et al., 2003). According to Kennedy et al. (1993), Zn from polysaccharide complex organic trace minerals may be more available to ruminant bacteria. When lactating dairy cattle were supplemented with a typical inorganic ZnO or half the Zn coming from ZnO and the other half in the form of Zn proteinate the cows supplemented with half the Zn from Zn proteinate had fewer new mammary infections (Spain et al., 1993). The advantage of organic Zn compared with inorganic Zn might have greater retention rather than improved absorption (Spears, 1989).

Toxicity

Some previous research indicated that early signs of Zn toxicity were reduced feed intake and body weight gain, more severe signs included diarrhea, lameness, internal hemorrhaging, and death (NASEM, 2016). According to the NASEM (2016), the maximum tolerable level for Zn is 500 mg/kg for any stage of beef cattle.

CONCLUSION

In conclusion, as modern technology and animal agriculture continue to develop, the beef cattle industry will develop livestock that will outperform their ancestors. As livestock agriculture continues to develop and focuses on greater levels of production, demands of producers and consumers will continue to evolve as well. With these constant demands, producers will have to be more efficient, but the same general structure of nutrition will remain. Livestock will still require the same nutrients, such as energy, water and protein. Mineral requirements will continually be adjusted to fit the demands of more-modern, advanced livestock, with more research needed to investigate how mineral supplementation affects animal longevity, production, and reproductive performance.

CHAPTER 2

Introduction

Mineral supplementation is a necessity in order to maintain optimal production in beef cattle. It has been shown that trace minerals, such as Zn, Mn, and Cu are necessary in beef cattle for optimal reproductive efficiency, maximal growth rate and rate of gain do, and adequate health and well-being. However, the source of trace minerals in the diet impact their bioavailability to the animal. Kegley and Spears (1994) found that an organic Cu source was equal to or more bioavailable than the inorganic Cu source, $CuSO_4$. Ahola et al. (2004) found that supplementing beef cows with organic proteinate sources of Cu, Zn, and Mn tended to increase pregnancy rates. Therefore, the purpose of the current study was to further investigate the effect of supplemental trace minerals as sulfates (Zn sulfate, Mn sulfate, and Cu sulfate) compared to hydroxychloride trace mineral sources (Zn hydroxychloride, Mn hydroxychloride, and basic Cu chloride; Micronutrients USA, Indianapolis, IN) on beef heifer development.

Materials and Methods

The University of Arkansas Animal Care and Use Committee (Protocol # 15066) approved all techniques and practices utilized in this study. During this study, 287 crossbred heifers, with an average weight of 255 ± 4.5 kg and an average age of 295 ± 16.5 d, were used over a 2-yr period. At the University of Arkansas Division of Agriculture's Beef Research Unit near Fayetteville, AR, 143 heifers were used; (n = 71 and 72 for breeding blocks 1 and 4; July 24, 2015 and July 28, 2016, respectively). At the University of Arkansas Division of Agriculture's Livestock and Forestry Research Station near Batesville, AR, 144 heifers were used; (n = 72 in each of breeding blocks 2 and 3; July 23, 2015 and December 15, 2015 respectively). Within each breeding block, heifers were stratified based on initial body weight, heifer age, heifer health, use in prior research projects, and heifer sire. Once stratified, heifers

were assigned to 6 groups of 12 heifers each, and these 6 groups were then assigned randomly to 1 of 2 trace mineral treatments. The 2 trace mineral treatments consisted of either sulfate or hydroxychloride trace minerals. Group integrity was maintained throughout the study's entirety at each research unit. All groups were maintained on similar fescue-bermuda grass mixed pastures and groups were rotated every 28 d among 2.4-ha pastures (n = 6), in order to minimize pasture effects.

Experimental Treatments. The 2 experimental treatments differed in the sources providing the trace minerals. Trace minerals were (Cu [74 mg/d], Mn [294 mg/d], and Zn [221 mg/d]) as 1) sulfate or 2) hydroxychloride sources (Micronutrients USA, Indianapolis, IN). These trace minerals were custom blended, by the University of Arkansas Animal Science Feed Mill, into a basal free choice mineral (Table 1; Nutrablend LLC, Neosho, MO), which was formulated for a consumption rate of 113 g/d. Grab samples (n = 6) were taken from the first, middle, and last 23-kg bag of each 182-kg batch of mineral mixed at the feed mill, and a composite sample was made of each treatment at each location as each bag was opened to be used (Table 1). When these free choice mineral treatments were provided in the pasture, they were placed in a 3-slot, weather protected, free-choice mineral feeder, in a single location relative to water and feed sources. Mineral feeders were checked daily and records were maintained of any mineral that was added to mineral feeders. When mineral disappearance exceeded the desired 113 g/d, the mineral feeders were moved away from water and feed sources, or salt content of the commercial free choice mineral was increased or decreased between batches with 12 or 20% salt content, to control mineral disappearance. Every 28 d, when it was time to rotate pastures, the mineral feeders were moved with their respective group of

heifers to the next pasture. Any remaining mineral was weighed on these days to calculate mineral disappearance for each period.

Records were kept as to where heifers were penned and pasture samples were collected every 28 d for nutrient analysis, which was achieved by taking grab samples (n = 6) from each pasture (Table 2). When farm managers and investigators subjectively determined pastures were not providing adequate forage, heifers were offered ad libitum access to 1.5 m by 1.5 m round bales of mixed fescue-bermuda grass hay. Samples were taken from every third bale when fed, for nutrient analysis (Table 3). Records were also kept on all corn gluten supplements, with samples being taken for nutrient analysis from every delivery (Table 4). Corn gluten supplements were offered to heifers for the entirety of the study, at 0.5% of their body weight. Finally, water sources consisted of automated waterers from wells. Water samples were taken once a year for mineral analysis (Table 5) at each research unit.

Heifer Growth and Health Measurements. At the beginning of the study, a 2-d consecutive weight was recorded (d -1 and d 0), and averaged for the initial weight. On d 0, hair coat scores were recorded described by Foster et al. 2016; Smith, 2016. During the study, body weights and hair coat scores were recorded every 28 d. At the end of the study (March 4, 2016; March 31, 2016; September 9, 2016; and March 9, 2017 for breeding blocks 1, 2, 3, and 4 respectively) a 2-d consecutive final weight (for an average final weight) was obtained. Body weights were obtained in the morning before corn gluten supplementation. Health status of each heifer was monitored for the entirety of each trial, and detailed health records were maintained. All heifers were treated for internal and external parasites, and vaccinated based on each research unit's own cattle management protocol.

Reproductive Data and Breeding Strategy. Two different breeding strategies were utilized. At the Fayetteville Unit, the breeding season was initiated on d 112 and d 105 for breeding blocks 1 and 4, respectively. At the start of breeding season, heifers were administered a 25 mg PGF2alpha injection (Lutalyse®, Zoetis, Parsippany, NJ) intramuscularly in the neck, and a heat detection patch (Estrotect Heat Patches®, Melrose, MN) was placed on top of each heifer's tail head. Also, using ultrasound a reproductive tract score was recorded for each heifer (Anderson et al., 1991). The presence of a corpus luteum was recorded, as well as whether heifers were cyclic at the time of breeding, and follicle size of those heifers that were cyclic.

Afterwards, these heifers were moved to 6, 1 ha grass pastures, along with their respective mineral feeders. During the following 7 d, 2 individuals monitored all heifers for estrus activity at 8:30 am and 4:30 pm. If estrus was detected, heifers were artificially inseminated within 12 to 18 h by 1 of 2 technicians. After 7 d of estrus detection, heifers that were not detected in estrus were administered a second injection of PGF2alpha, and observed for estrus for an additional 5 d. Once this was completed, all heifers were moved, along with their respective mineral feeders, back to their assigned 2.4-hectare pastures. Seven days following the transfer back to the study pastures, 6 fertile bulls, tested within 30 d of the start of breeding season, were used for a 50-d breeding season. During the breeding season, bulls were rotated between replicate pastures every 7 d. At the end of the 50-d breeding season, all bulls had a breeding soundness examination completed and were found to be reproductively sound.

At the Batesville research unit, the breeding season was initiated on d 130 and d 146 for breeding blocks 2 and 3. At this research unit, no artificial insemination was conducted. Six fertile bulls were used for a 60-d breeding season. During the breeding season, bulls were rotated

between pasture replicates every 7 d. At the end of the 60-d breeding season, all bulls passed another breeding soundness examination and were reproductively sound.

Pregnancy rates were determined differently at each research unit. At the Fayetteville Research Unit, ultrasound observations were utilized to determine pregnancy by artificial insemination, on d 168 for blocks 1 and 4. Then, at both locations at the conclusion of each trial, final pregnancy status was determined by the presence of Pregnancy-Specific Protein B concentrations in blood (B & D Genetics, Cherry Valley, AR).

Calf Measurements. At the completion of each trial, open heifers were culled and pregnant heifers were managed as one group. During this time, heifers were supplemented with adequate free choice mineral supplements and were grazed on fescue-bermuda grass pastures. At calving (within 24 h of birth) calves were weighed and calf sex recorded.

Laboratory Analyses. Forage, hay, and corn gluten feed samples were dried in a forced air oven at 50 °C and ground through a 1 mm screen in a Willey Mill (Arthur H Thompson, Phil., PA). Forage, hay, and corn gluten feed samples were analyzed for DM, ash, ADF and NDF concentrations (Ankom Fibers, Van Soast 25 Method), percentage nitrogen for crude protein percentage (Rapid combustion AOAC 1998 method 990.03, Elementar Americas Inc., Mt. Laurel, NJ), and mineral concentrations. Free choice mineral grab samples at each research unit and first, middle, and last bag mineral batch samples were analyzed for mineral concentrations (Table 5). Mineral analyses were done in duplicate for forage, hay, and corn gluten feed samples and in triplicate for first, middle, last bags and mineral batch grab samples. Analysis began with weighing 1 ± 0.01 g of grass, hay, and corn gluten feed sample or 0.5 ± 0.01 g of free choice mineral, into a 50 mL centrifuge tube, and adding 15 mL of trace mineral grade nitric acid (9598-34 , J.T. Baker, Phillipsburg, NJ). The wet ash digestion was done by covering the tubes

with plastic watch glasses and placing them into a heating block. The temperature was set at 80 °C for 15 min, or until all brown gasses had escaped and no foaming was occurring. After brown gasses had escaped the temperature was reset to 115 °C for 1 h. After the 1 h, tubes were allowed to cool and were filled to a 45 mL volume with deionized water, inverted, and capped. Samples were analyzed by inductively coupled plasma (ICP) atomic emission spectroscopy (CIROS, Fitchburg, MA) at the University of Arkansas, Fayetteville, AR (Altheimer Laboratory). Water samples were also analyzed as is by ICP (Arkansas Water Resources Central Analytical Laboratory, Fayetteville, AR).

Liver biopsy samples were collected from select heifers (n = 24/breeding block) at the beginning and end of each research trial. For each breeding block, 4 heifers/pasture were randomly stratified to be collected for liver samples based on initial body weight, heifer age, heifer health, use in prior research projects, and heifer sire. Animals were restrained in a hydraulic squeeze chute, and the 10th intercostal space on right side of the animal was identified on the abdomen. An area approximately 10 cm by 10 cm was clipped using an electric clipper. Using aseptic technique, the area was then scrubbed using chlorhexidine gauze sponges followed by scrubbing with 70% isopropyl alcohol gauze sponges and a final single scrub of iodine surgical solution. The site of incision was then injected with 5 mL of 2% lidocaine solution (Phoenix Lidocaine Hydrochloride Injectable - 2%, Clipper Distributing, Inc., St. Joseph, MO) under the skin and into the intercostal muscle. After allowing 5 min wait period for lidocaine to take effect within the surgical area, a sterile #15 scalpel was used to make a 1 cm incision through the skin. A Tru-Cut Style biopsy needle (Cardinal Health, Dublin, OH) was then inserted through the incision to obtain liver samples. The same biopsy needle was used to obtain multiple samples from the same heifer, until at least a 0.05 g sample was obtained. A sterile transfer pipet

was used to remove liver from the biopsy needle and samples were placed in acid-washed, pre-weighed borosilicate tubes, covered with parafilm, and stored on ice. In the laboratory, liver samples were weighed before placing into a forced air oven to be dried at 100 °C for 48 h, to remove all remaining moisture. Once dried, samples were weighed to record a dried sample weight. Samples were then placed into a modified heating block and 2 mL of trace mineral grade nitric acid (9598-34, J.T. Baker, Phillipsburg, NJ) was added. The wet ash digestion was done by covering the test tubes with a glass marble and the temperature was set at 80 °C for 15 min, or until all brown gasses had escaped and no foaming was occurring. After brown gasses had escaped the temperature was reset to 115 °C for 1 h. Tubes were allowed to cool, were filled to a 5 mL volume by weight with deionized water, vortexed, and poured into a scintillation bottle. Samples were analyzed by ICP (CIROS, Fitchburg, MA) at the Altheimer Laboratory, University of Arkansas, Fayetteville, AR, in which samples were compared with certified bovine liver samples (NIST, Washington, DC) as a standard.

Plasma samples were collected from all heifers at the beginning, pre-breeding, and at the end of each research trial, via jugular venipuncture in 7 ml sodium heparin treated trace element vacuum tubes (Kendall, Mansfield, MA). Samples were transported on ice to the University of Arkansas Animal Science Nutrition Lab, where they were centrifuged at 1,200 x g for 20 min. Plasma was removed and stored at -20 °C, until analysis. Prior to analysis, samples were thawed at room temperature and vortexed to mix thoroughly. A 500 µl aliquot of plasma was diluted with 9.5 mL of trace mineral grade nitric acid in a 15 mL centrifuge tube. Following at least a 24 h incubation at room temperature, the samples were centrifuged at 1,000 g for 20 min and the aliquot was harvested from the pellet and analyzed by ICP (CIROS, Fitchburg, MA) at the Altheimer Laboratory, University of Arkansas, Fayetteville, AR.

Statistical Analyses. Data were analyzed in a randomized complete block design with pen as the experimental unit. The model included breeding block as a random effect and treatment as a fixed effect. All data were analyzed using various procedures in SAS (SAS Inst., Inc., Cary, NC). Heifer weights, average daily gains, and hair coat scores were analyzed using the MEANS procedure for overall pen average. Heifer weights and hair coat scores were analyzed using the MIXED procedure, with the model including treatment, day, and the treatment by day interaction, with day as a repeated measure. Average daily gains were analyzed using PROC MIXED, with model including treatment as the fixed effect and block in the random statement. All morbidity data, which included antibiotic given for respiratory illness, pinkeye occurrence, footrot occurrence, and overall health of the heifers were analyzed using PROC GLIMMIX, with model including treatment as a fixed effect and block in the random statement. Mineral disappearance was calculated as grams per head per day and overall mineral disappearance during the study was analyzed using PROC MIXED, with model including treatment, period, and treatment by period interaction for grams per heifer per day and treatment for overall mineral disappearance. Liver trace mineral concentrations for Zn, Cu, and Mn, and plasma trace mineral concentrations for Zn, Cu, and Mn were all analyzed using PROC MIXED, with the model including treatment, period, and treatment by period interaction, with period as a repeated measure. Finally, reproductive data were analyzed using the Chi Square procedure with *P* values being generated from the GEN MOD model, and the model including block and treatment in the class statement. Forage, hay, feed supplements, water, and free choice mineral sample data were generated using the PROC MEANS procedure. Significance was determined when $P \leq 0.05$, with tendencies determined when $P > 0.05$ and ≤ 0.10.

CHAPTER 3

Results and Discussion

Heifer Performance. Heifer body weights did not differ due to treatment during the study ($P = 0.77$). There was a day effect for body weight ($P < 0.0001$) during the study, with heifers increasing in body weight from the initiation of the study until the conclusion of the study. Also, there was not a treatment x day interaction ($P = 0.99$; Figure 1). When comparing average daily gains of heifers from sulfate and hydroxychloride treatments, ADG was similar ($P \geq 0.52$) with both groups gaining on average 0.58 kg/d (Figure 2). In addition, hair coat scores were similar ($P = 0.99$) between treatments and there was no treatment × day interaction ($P = 0.76$); however, there was a day effect ($P < 0.0001$; Figure 3). This finding is possibly due to the different weigh days when hair coat scores were taken, since breeding blocks 1, 2, and 4 were taken during the fall season, and breeding block 3 hair scores were taken during the spring season.

An Arthington and Swenson (2004) study using Braford cows and supplementing inorganic sulfate trace minerals or organic amino acid complexed sources of Zn, Cu, and Mn, reported similar findings with the current study indicating that source of trace mineral had no effect on body weight. Ahola et al. (2005) also reported similar findings in a heifer study supplementing inorganic sulfate sources and organic proteinate sources of Zn, Cu, and Mn, indicating no differences in body weight, and no treatment by time interaction. This is also in agreeance with several other previous experiments that reported that trace mineral source or supplementation had an effect on cow body weight (Olson et al., 1999; Stanton et al., 2000).

Mineral Disappearance. Even with the forages being deficient in Cu, these supplements exceeded NASEM (2016) requirements. We found after analyzing the 9, 28-d periods for mineral that was missing from the free-choice mineral feeders, there were no differences

between treatments ($P = 0.46$) or the treatment by period interaction ($P = 0.99$). However, there was a period effect ($P = 0.02$), when comparing period 4, with the lowest mineral disappearance rate, to all other periods in the study (Figure 4). With this finding, the average mineral disappearance of each trace mineral formulation was 102 g/d compared to 109 g/d for hydroxychloride and sulfate trace mineral sources respectfully.

The current study results differed from a recent study with pre- and post-weaned beef calves, which reported that calves consumed more mineral containing hydroxychloride compared to either organic or sulfate trace mineral sources of Zn, Cu, and Mn (Caramalac et al., 2017). This previous study is supported by a study in swine, which indicated that diets formulated with basic Cu chloride were consumed more preferentially compared to diets formulated with Cu sulfate (150 mg Cu/kg diet; Coble et al., 2014). Caramalac et al. (2017) suggested that increased intake of hydroxychloride trace mineral supplements was due to the lower solubility and higher palatability of this source compared to the metallic taste of organic and sulfate sources, which could act as a possible taste aversion.

Heifer Health. There were no differences found between sulfate and hydroxychloride treatment heifers in the need for antibiotic treatment for respiratory disease, pinkeye, footrot, or other health problems (Table 6). The incidences of bovine respiratory disease were also not affected by treatment ($P = 0.77$) with 4.2% of heifers being treated in the sulfate groups and 5.6% of heifers being treated in the hydroxychloride groups. A similar 1.4% of heifers were treated for pinkeye in the sulfate groups compared to 5.6% heifers from the hydroxychloride groups ($P = 0.20$). In the case of those heifers that were treated for foot-rot, there were only 1.4% of heifers in the sulfate groups and 0.7% of heifer from the hydroxychloride groups ($P = 0.57$).

Finally, the total amount of medical treatments ($P = 0.74$) were 4.9% of heifers were treated in the sulfate mineral treatments and 6.3% of heifers were treated in the hydroxychloride groups.

Ryan et al. (2015) reported similar findings indicating that mineral source, whether it was organic, sulfate, or hydroxychloride, had no effect on the percentage of receiving cattle treated for bovine respiratory disease with an overall 62% treatment rate for bovine respiratory disease in highly stressed steers. In addition, Kegley et al. (2012) reported no differences for the total number of high risk beef calves treated for bovine respiratory disease that had organic and inorganic trace mineral supplements ($P = 0.46$).

Reproductive Performance. There was no difference in overall pregnancy rate between treatments ($P = 0.85$) with 84.6% vs. 85.4% of heifers being confirmed pregnant in the hydroxychloride and sulfate treatments, respectively (Table 7). Furthermore, bullbred pregnancy rates ($P = 0.71$; 70% and 71.5%) and artificial insemination pregnancy rates ($P = 0.83$; 29.4% versus 27.8%) were similar between treatments. Of the heifers that were confirmed pregnant at the conclusion of the treatment feeding period in blocks 1, 2, and 3; 82.4% of the hydroxychloride treatment calved compared to 81.5% of the sulfate treatment. After blocks 1, 2, and 3 calved, there was no difference ($P = 0.97$) for mean calf birth weight (32.3 kg versus 32.2 kg) for heifers in the hydroxychloride compared to the sulfate treatment groups.

During this study, there were additional data measurements taken at the Fayetteville Research Unit, which included ultrasound data for the presence of a corpus luteum, those heifers that were cyclic at the time of breeding, and follicle size of those heifers that were cyclic. Treatment had no effect ($P = 0.55$; 50% of the hydroxychloride heifers vs 45.1% of the sulfate heifers) on a corpus luteum being present at the start of the breeding season. Furthermore treatment had no effect ($P = 0.44$) on those heifers being cyclic at the start of breeding. Finally,

treatment had no effect (P = 0.63) on the follicle size of those heifers that were determined to be cyclic.

Whitehurst et al. (2014) reported contrary results from the above data, indicating that methionine hydroxy analog chelate form of trace minerals improved overall pregnancy rates (81% vs. 79%) when compared to sulfate trace mineral sources. However, Ahola et al. (2005) reported data that indicated overall pregnancy rates tended to be greater (97.6% vs. 86.5%) for heifers fed inorganic sulfate trace mineral sources compared to those organic proteinate trace mineral sources. Ahola et al. (2004) reported findings in multiparous cows, indicating no differences in pregnancy rates (95% vs. 93%) for cows consuming inorganic sulfate trace mineral compared to organic proteinate trace mineral sources. Ahola et al. (2004 and 2005) also reported no differences between inorganic sulfate and organic proteinate trace mineral supplementation sources in beef cows and heifers

Ahola et al. (2004) supports data from the current study indicating that there were no differences between multiparous cows that were cyclic consuming organic and inorganic trace mineral supplementation. The authors also reported no differences in observed estrus after PGF2alpha injection for cows consuming organic proteinate and inorganic sulfate trace mineral supplementation. However, Ahola et al. (2005) reported that beef heifers consuming organic proteinate trace mineral supplementation tended to have a greater percentage of heifers cyclic at the time of breeding compared to inorganic sulfate trace mineral supplements, but showed no differences between mineral treatments when estrus was observed after PGF2alpha administration.

Liver Mineral Concentration. Liver Cu concentrations were not affected by treatment (P = 0.98) or a treatment by period interaction (P = 0.23). There was an effect for period (P = 0.02),

with heifers on both sulfate and hydroxy treatments exhibiting an increase in liver Cu concentrations (143 to 172 mg/kg) over the length of the study (Figure 5). Based on ranges from Kincaid (2000), the adequate Cu concentration in the liver is 125 to 600 mg/kg. These heifers maintained liver Cu concentrations within the adequate range during this project. This suggests that the basic Cu chloride provided heifers with efficiently absorbed Cu from the free choice mineral as well as the Cu sulfate. In addition, McDowell (1992) stated that the concentration of Cu in the liver of ruminants is correlated to the bioavailability of the Cu in the diet. Caramalac et al. (2017) reported similar findings, indicating that when different Cu sources, such as basic Cu chloride, Cu sulfate, or when no trace mineral supplementation was provided, were compared in steers, the liver Cu concentrations were not different between treatments. Ahola et al. (2005) also found that liver Cu concentrations increased from the beginning to the end of their study, but found the organic proteinate treatments had greater Cu concentrations in the liver compared to inorganic treatments.

There was no treatment difference ($P = 0.81$) found for liver Zn concentration or for a treatment by period interaction ($P = 0.60$). However, there was a period effect ($P < 0.0001$), with sulfate and hydroxychloride Zn sources increasing the liver Zn concentration an average of approximately 50 mg/kg from the beginning to the end of the study (95 and 142 mg/kg; Figure 6). Kincaid et al. (1976) reported that the relationship between Zn intake and concentration of Zn in the liver is affected by age of the ruminant. Since in the current study heifers were still growing and not fully mature, liver Zn concentration was more likely to increase than compared to a mature cow. Based on Kincaid (2000), the adequate range of Zn concentration in the liver is 25 to 200 mg/kg. These heifers' liver Zn concentrations were within the adequate range throughout the trial. In addition, since liver Zn concentrations did not differ due to treatment, this

suggests that the Zn hydroxychloride source was as bioavailable as the Zn sulfate for beef cattle. Caramalac et al. (2017) reported similar findings in liver Zn concentrations for steers fed hydroxychloride, sulfate, or no trace mineral supplementation at all, and found no difference between treatments. During a 2-yr cow study, Ahola et al. (2004) found in year one that there were no differences between organic and inorganic mineral treatments when looking at liver Zn concentrations, but inorganic cows tended to have greater liver Zn concentrations at the end of the second year

Kincaid (2000) suggested, that Mn is taken up by the liver and excreted via bile, so accumulations of Mn in the liver often do not reflect dietary intake of Mn. However, Kincaid (2000) also suggests that the liver Mn concentration in cattle should be between 7 mg/kg and 15 mg/kg. When beef heifers consuming organic and inorganic treatments were compared by Ahola et al. (2005), organic sources tended to have greater concentrations of Mn in liver tissue. Caramalac et al (2017), reported that there was no difference in liver Mn concentration due to treatment of (P = 0.83) hydroxychloride, sulfate, and organic mineral. Even with these above findings, with our current research we found that Mn levels in liver tissue samples were below the detection limit of 0.01 mg/L.

Plasma Mineral Concentrations. There was no effect of treatment (P = 0.77) or a treatment by period (P = 0.97) interaction for plasma Cu concentrations (Figure 7). There was a period effect (P <0.0001) with the greatest plasma Cu concentrations being at pre-breeding and the lowest being at the start of the study. According to ranges from Kincaid (2000), these heifers started the study with an adequate plasma Cu concentration (0.7 to 0.9 mg/L) and finished with high concentrations of Cu in plasma for beef cattle (0.9 to 1.1 mg/L).

There were no effects of treatment ($P = 0.87$) or a treatment by period interaction ($P = 0.71$) on plasma Zn concentrations (Figure 8). There was a period effect ($P < 0.0001$) with the greatest plasma Zn concentrations at the end of the study (1.81 mg/L) and the lowest plasma Zn concentrations being at the start of the study (1.7 mg/L). According to reference ranges from Kincaid (2000), beef heifers should have been within a range of 0.8 to 1.4 mg/L. Therefore, the study heifers would be considered above normal range for plasma Zn concentrations.

Implications

Based on the current study, growth, health, and reproductive performance for beef heifers were similar for sulfate and hydroxychloride trace mineral treatments. This suggest that supplementing with either sulfate or hydroxychloride sources of Zn, Cu, and Mn to developing beef heifers should result in similar growth and reproductive performance. The next step to further investigate differences between sources of supplemental Zn, Cu, and Mn would be to use a fixed timed artificial insemination breeding strategy, in hopes that breeding at 72 to 80 h later would show differences in that heifers that are reproductive sound will breed sooner than those heifers that have not reached puberty yet based on mineral supplementation treatments.

Table 1. Ingredient and nutrient composition of free choice mineral supplements

	Sulfate		Hydroxychloride	
Ingredient	%			
Salt	20	12	20	12
Dicalcium phosphate, 18.5% P	32.4		32.4	
Calcium carbonate	27.5		27.5	
Magnesium oxide, 54% Mg	3.7		3.7	
Molasses and grain products	13	21	14	22
Mineral oil	1		1	
Sodium selenite, 0.4% Se	0.5		0.5	
Calcium iodate	0.01		0.01	
Cobalt carbonate, 46% Co	0.01		0.01	
Vitamin premix[1]	0.12		0.12	
Copper sulfate, 25% Cu	0.26		-	
Zinc sulfate, 35.5% Zn	0.55		-	
Manganese sulfate, 32% Mn	0.81		-	
Basic copper chloride, 58% Cu	-		0.11	
Zinc hydroxychloride, 55% Zn	-		0.35	
Manganese hydroxychloride, 44% Mn	-		0.59	
Analyzed Composition[2]				
P, %	4.7 ± 0.12		5.2 ± 0.10	
K, %	0.30 ± 0.01		0.31 ± 0.01	
Ca, %	16.9 ± 0.26		16.8 ± 0.38	
Mg, %	1.91 ± 0.04		1.72 ± 0.05	
S, %	0.64 ± 0.02		0.43 ± 0.01	
Na, %	7.6 ± 0.23	5.2 ± 0.11	7.2 ± 0.26	4.8 ± 0.13
Fe, mg/kg	3,405 ± 96		3,649 ± 109	
Mn, mg/kg	3,017 ± 132		2,682 ± 121	
Zn, mg/kg	1,432 ± 118		1,572 ± 107	
Cu, mg/kg	880 ± 46		609 ± 81	
Co, mg/kg	17.3 ± 3.1		19.0 ± 3.1	
B, mg/kg	11.6 ± 0.26		12.4 ± 0.65	

[1]Vitamin Premix contained 220,462 IU/kg Vitamin A, 22,046 IU/kg Vitamin D, and 220 IU/kg Vitamin E

[2]SEM is calculated by PROC MEANS of SAS, with averages between batches

Table 2. Nutrient composition of forage samples from pastures[1]

Block[2]	1	4	2	3
		- - - - - - - -% - - - - - - - -		
DM	97.14 ± 0.73	97.47 ± 0.31	95.20 ± 2.58	97.90 ± 0.39
CP	10.38 ± 1.66	11.35 ± 1.78	15.94 ± 3.13	16.52 ± 4.42
NDF	69.17 ± 3.45	68.27 ± 6.64	62.56 ± 4.46	58.78 ± 6.46
ADF	34.31 ± 2.82	36.18 ± 1.80	28.73 ± 2.70	30.77 ± 5.42
Ash	7.35 ± 0.92	9.93 ± 1.21	9.66 ± 1.32	10.63 ± 1.34
P	0.26 ± 0.07	0.30 ± 0.05	0.32 ± 0.04	0.29 ± 0.07
K	1.17 ± 0.66	1.22 ± 0.49	2.40 ± 0.63	2.48 ± 0.69
Ca	0.41 ± 0.06	0.46 ± 0.02	0.59 ± 0.10	0.45 ± 0.07
Mg	0.16 ± 0.04	0.18 ± 0.02	0.22 ± 0.04	0.21 ± 0.03
S	0.17 ± 0.05	0.21 ± 0.03	0.21 ± 0.01	0.21 ± 0.03
Na	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01
		- - - - - - - - mg/kg - - - - - - - -		
Fe	192.40 ± 124.84	231.05 ± 113.22	105.83 ± 6.50	143.23 ± 76.67
Mn	138.10 ± 14.19	119.01 ± 14.20	95.65 ± 14.57	106.21 ± 21.92
Zn	56.11 ± 11.70	65.54 ± 12.50	29.21 ± 2.11	16.09 ± 3.27
Cu	6.04 ± 0.92	6.89 ± 0.66	7.13 ± 1.22	4.80 ± 1.02
Co	0.01 ± 0.01	0.15 ± 0.08	0.05 ± 0.00	0.09 ± 0.08
B	2.92 ± 1.07	3.62 ± 0.41	3.72 ± 0.52	3.17 ± 0.98

[1]Mean ± standard deviation; n = 10

[2]Block 1: First heifer set at Fayetteville (July 24, 2015 to March 4, 2016); Block 4: Second heifer set at Fayetteville (July 28, 2016 to March 9, 2017); Block 2: First heifer set at Batesville (July 23, 2015 to March 31, 2016); Block 3: Second heifer set at Batesville (December 15, 2015 to September 9, 2016)

Table 3. Nutrient composition of hay offered[1]

Block[2]	1	4	2	3[3]
		- - - - - - - -% - - - - - - - -		
DM	97.56 ± 0.14	97.55 ± 0.39	97.87 ± 0.41	.
CP	11.06 ± 2.23	23.77 ± 8.27	9.01 ± 1.53	.
NDF	71.20 ± 0.96	69.05 ± 1.10	70.90 ± 2.26	.
ADF	41.15 ± 2.39	34.58 ± 4.30	43.32 ± 4.91	.
Ash	8.02 ± 1.07	11.71 ± 1.19	6.75 ± 0.64	.
P	0.38 ± 0.04	0.43 ± 0.02	0.31 ± 0.05	.
K	1.54 ± 0.14	1.96 ± 0.30	1.40 ± 0.37	.
Ca	0.55 ± 0.11	0.68 ± 0.08	0.61 ± 0.09	.
Mg	0.32 ± 0.08	0.35 ± 0.01	0.25 ± 0.08	.
S	0.19 ± 0.02	0.27 ± 0.03	0.10 ± 0.02	.
Na	0.04 ± 0.02	0.07 ± 0.02	0.01 ± 0.00	.
		- - - - - - - - mg/kg - - - - - - - -		
Fe	178.8 ± 76.50	189.91 ± 71.22	96.20 ± 24.07	.
Mn	106.1 ± 30.52	75.84 ± 25.99	135.03 ± 41.27	.
Zn	51.40 ± 7.50	54.53 ± 9.85	22.44 ± 3.24	.
Cu	8.88 ± 1.79	11.49 ± 2.08	5.79 ± 0.62	.
Co	0.10 ± 0.04	0.13 ± 0.05	0.07 ± 0.06	.
B	4.03 ± 0.97	5.25 ± 0.47	2.91 ± 0.44	.

[1]Mean ± standard deviation; n=2

[2]Block 1: First heifer set at Fayetteville; Block 4: Second heifer set at Fayetteville; Block 2: First heifer set at Batesville; Block 3: Second heifer set at Batesville

[3]Block 3 (Batesville) did not receive any hay supplementation during the entirety of the study

Table 4. Nutrient composition of corn gluten supplement[1,2]

Block[3]	1	4	2	3
		- - - - - - - -% - - - - - - - -		
DM	94.31 ± 0.55	96.34 ± 0.61	95.83 ± 2.51	96.74 ± 0.87
CP	21.19 ± 1.73	24.17 ± 2.16	22.36 ± 2.86	23.93 ± 1.11
NDF	40.22 ± 0.98	41.57 ± 3.34	37.59 ± 5.83	44.36 ± 2.25
ADF	10.96 ± 1.44	10.57 ± 0.62	9.62 ± 1.88	11.36 ± 0.85
Ash	6.72 ± 1.00	8.43 ± 1.50	5.97 ± 0.46	7.16 ± 0.91
P	1.06 ± 0.00	1.20 ± 0.08	1.12 ± 0.02	1.19 ± 0.05
K	1.45 ± 0.01	1.64 ± 0.29	1.57 ± 0.10	1.58 ± 0.08
Ca	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Mg	0.39 ± 0.01	0.42 ± 0.05	0.28 ± 0.03	0.37 ± 0.02
S	0.35 ± 0.02	0.49 ± 0.16	0.36 ± 0.07	0.50 ± 0.03
Na	0.25 ± 0.02	0.21 ± 0.01	0.26 ± 0.02	0.25 ± 0.01
		- - - - - - - - mg/kg - - - - - - - -		
Fe	115.38 ± 32.20	114.14 ± 33.13	97.78 ± 35.45	104.36 ± 25.09
Mn	21.80 ± 3.85	19.0 ± 2.82	18.35 ± 2.13	18.23 ± 1.18
Zn	72.51 ± 12.73	71.78 ± 13.12	68.48 ± 9.32	61.34 ± 4.31
Cu	6.56 ± 0.93	5.58 ± 0.96	5.75 ± 0.75	5.40 ± 0.43
Co	0.09 ± 0.01	0.08 ± 0.02	0.08 ± 0.01	0.09 ± 0.01
B	7.16 ± 0.04	7.70 ± 0.80	7.05 ± 0.45	6.92 ± 0.50

[1]Value ± standard deviation; n = 10

[2]Rate fed = 0.5 % of body weight/d

[3]Block 1: First heifer set at Fayetteville; Block 4: Second heifer set at Fayetteville; Block 2: First heifer set at Batesville; Block 3: Second heifer set at Batesville

Table 5. Mineral composition of water; mg/L[1]

Location	Fayetteville	Batesville
Ca	67.31 ± 19.29	70.82 ± 18.75
Mg	1.71 ± 1.19	1.82 ± 1.20
S	9.22 ± 1.25	12.17 ± 1.10
Na	14.59 ± 3.22	15.27 ± 2.89
Fe	0.00 ± 0.00	0.00 ± 0.00
Mn	0.00 ± 0.00	0.00 ± 0.00
Zn	0.01 ± 0.01	0.03 ± 0.01
Cu	0.26 ± 0.01	0.24 ± 0.01
Se	0.01 ± 0.01	0.01 ± 0.01
Cl	24.33 ± 5.81	6.40 ± 1.90

[1]Mean ± standard deviation; n = 2/location

Table 6. Comparison of heifer health treatment events[1]

	Hydroxychloride	Sulfate	SEM	*P* - value[2]
Respiratory treatment events, %	5.60	4.20	0.75	0.77
Pinkeye treatment events, %	5.60	1.40	1.09	0.20
Foot-rot treatment events, %	0.70	1.40	1.23	0.57
Overall treatment events, %	6.30	4.90	0.66	0.74

[1]SEM is calculated by PROC MEANS of SAS, with averages between treatments
[2]Calculated using PROC GLIMMIX of SAS

Table 7. Reproductive performance

	Hydroxychloride	Sulfate	SEM	*P*-Value
Pregnant, %	84.60	85.40		0.85
Heifers confirmed bullbred, %	70.00	71.53		0.71
A.I. exposed[1], %	63.40	65.30		0.82
A.I. confirmed[1], %	29.40	27.80		0.83
Reproductive tract scores	3.53	3.39	0.15	0.39
Corpus luteum present[1], %	50.00	45.10		0.55
Cyclic[1], %	54.30	47.90		0.44
Follicle size[1,2]	1.7	1.63	0.14	0.63
Calved[3], %	82.40	81.50		0.90
Calf birth weight, kg[2]	32.3	32.2	1.12	0.97
Loss pregnancy[2], %	6.50	4.60		0.51

[1]Only those heifers in breeding groups 1 and 4 at Fayetteville are included in this data

[2]Follicle size: 1 = follicle that is ≤ 5mm; 2 = follicle size that is 5 - 10 mm; and 3 = follicle size that is > 10 mm

[3]Only those heifers in the first three breeding groups (breeding groups 1, 2, and 3)

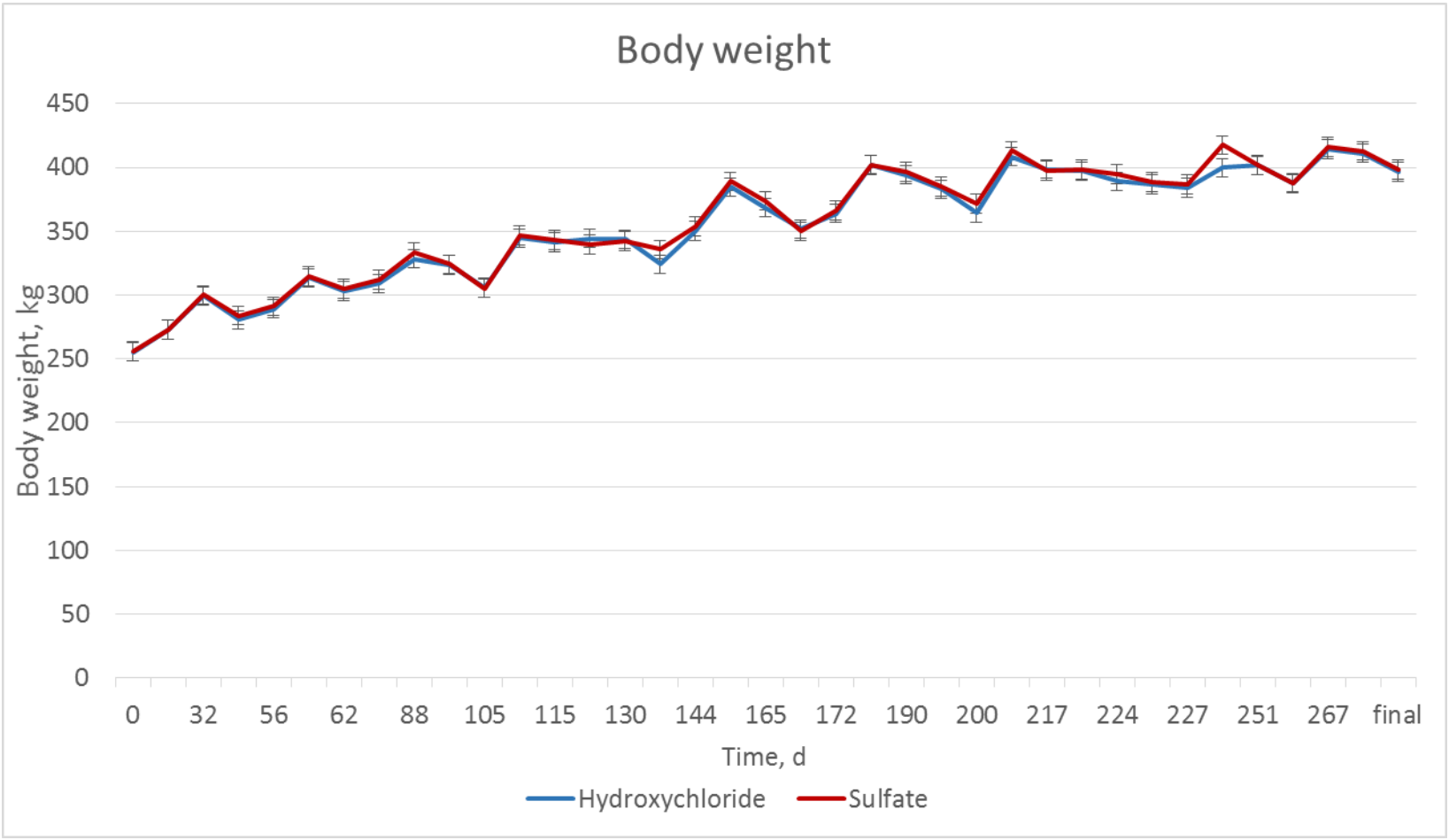

Figure 1. Mean body weight. Treatment ($P = 0.77$); Day ($P < 0.0001$); Treatment x Day ($P = 0.99$)

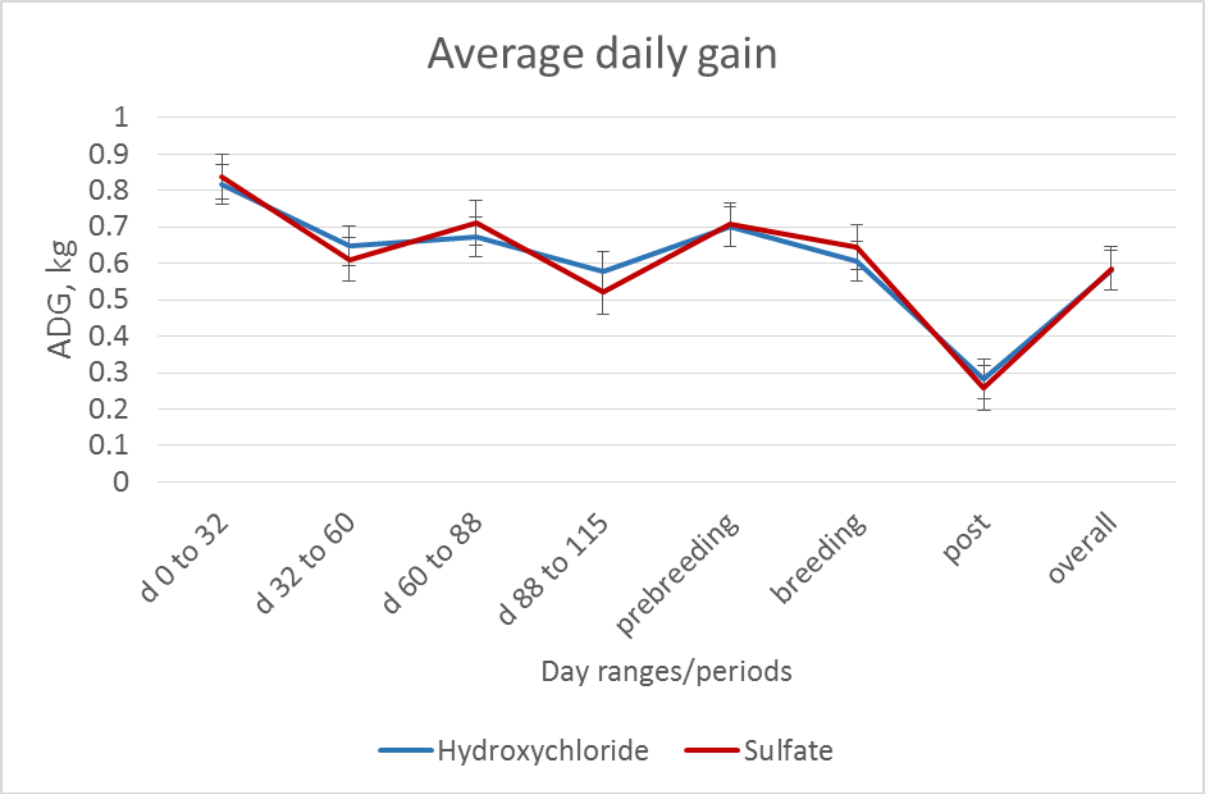

Figure 2. The average daily gains of heifers. Treatment ($P \geq 0.52$)

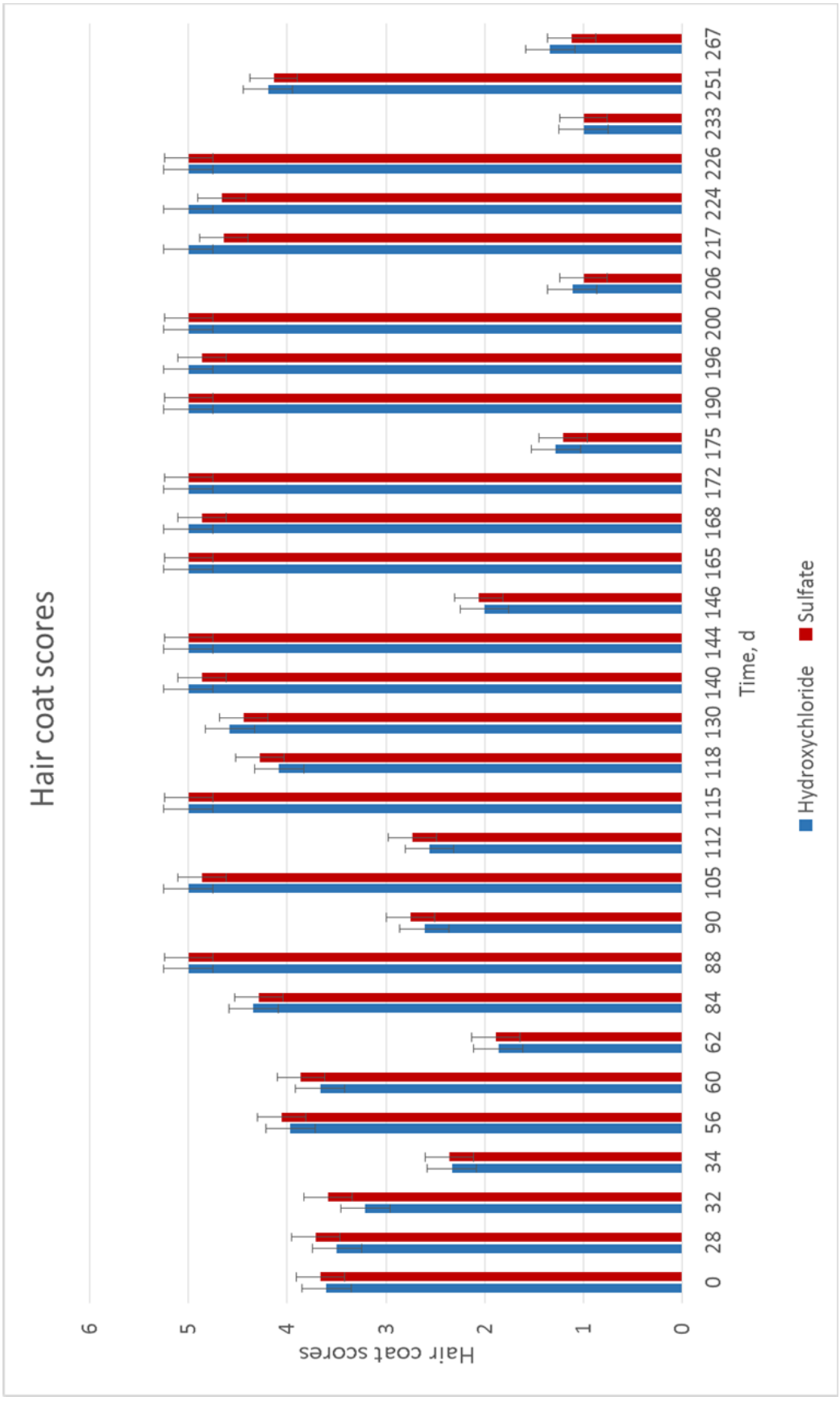

Figure 3. Hair coat scores of heifers. Treatment ($P = 0.99$); Period ($P < 0.0001$); Treatment x Period ($P = 0.76$)

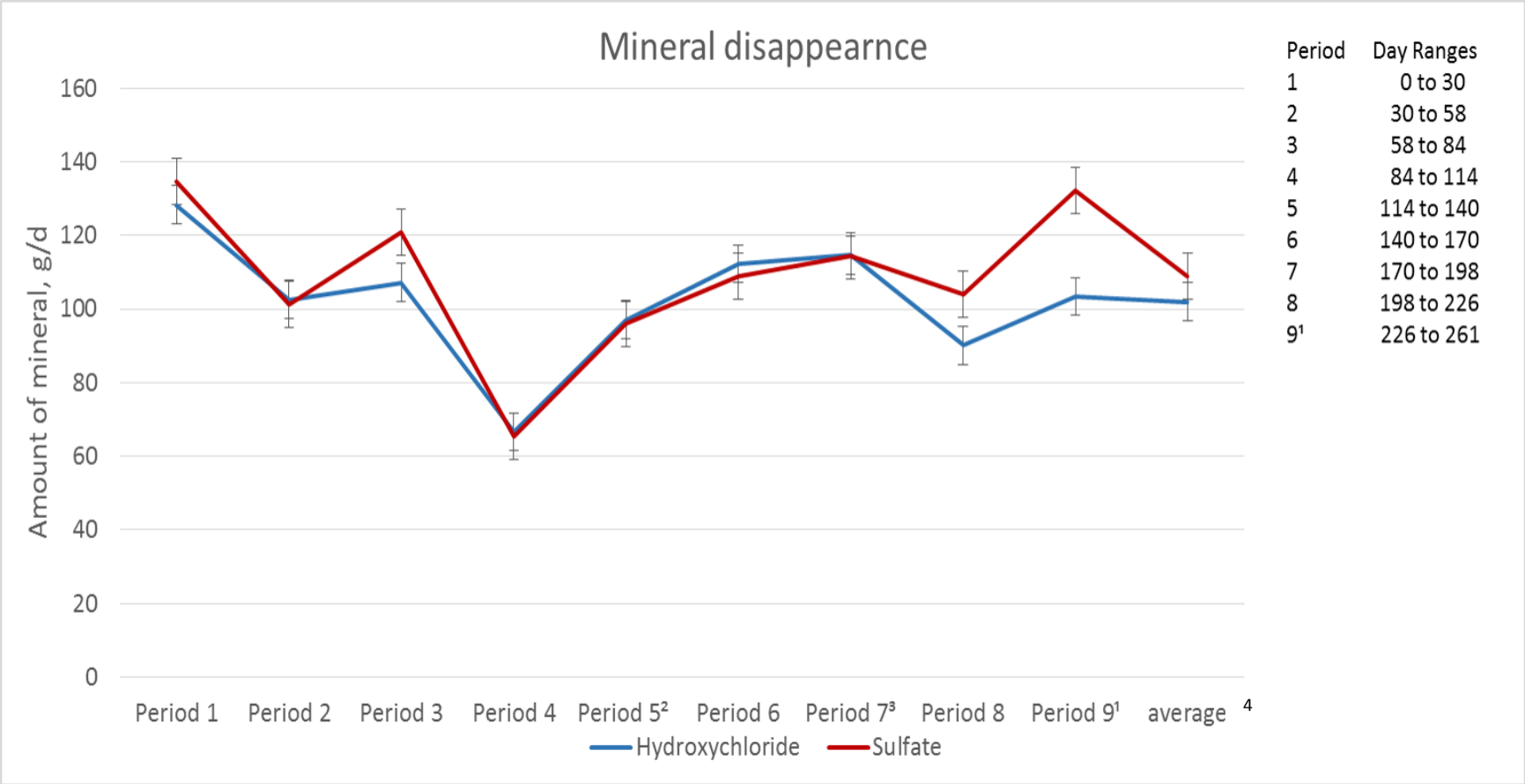

Figure 4. The mineral disappearance over time. Treatment (P = 0.46); Period (P = 0.02); Treatment x Period (P = 0.99)

[1]Only includes blocks at the Batesville Research Unit

[2]Beginning of breeding season

[3]End of breeding season

[4]Average disappearance over the study

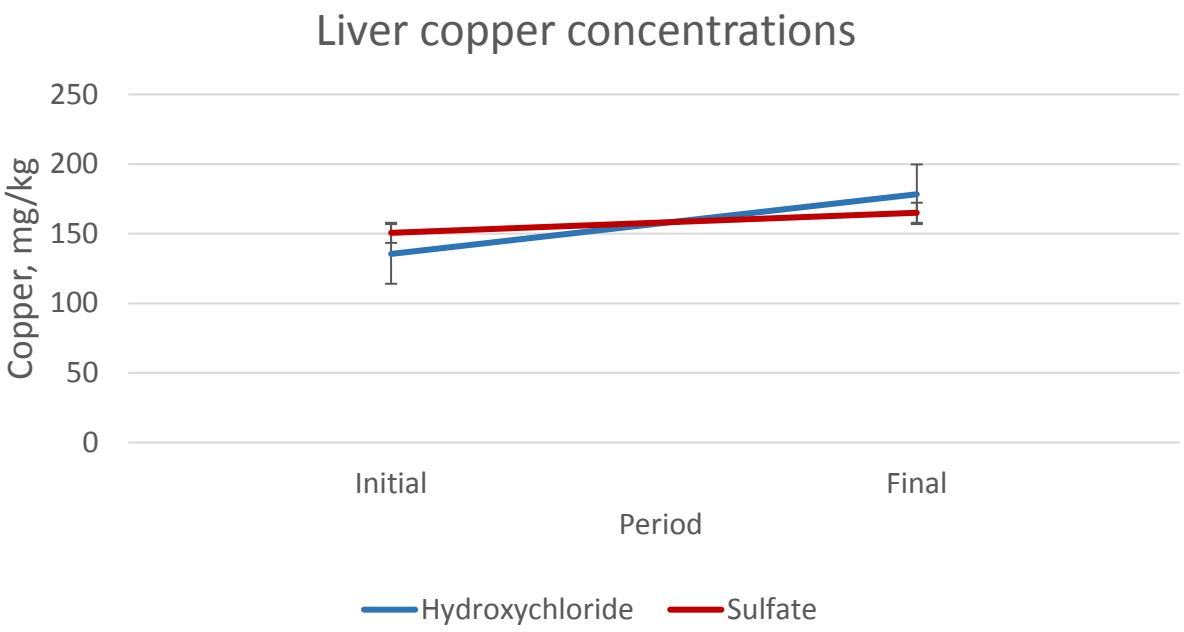

Figure 5. Liver copper concentration, mg/kg dry tissue. Treatment (P = 0.98); Period (P = 0.02); Treatment x Period (P = 0.23)

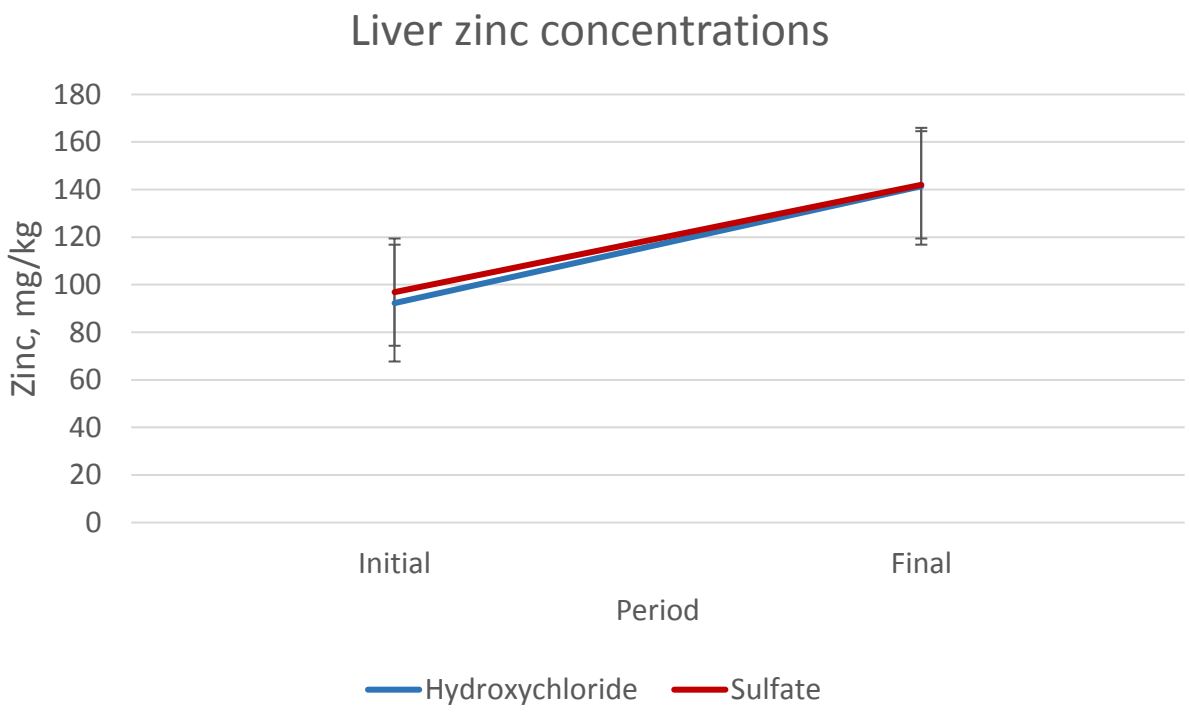

Figure 6. Liver zinc concentration, mg/kg dry tissue. Treatment (P = 0.81); Period (P < 0.0001); Treatment x Period (P = 0.60)

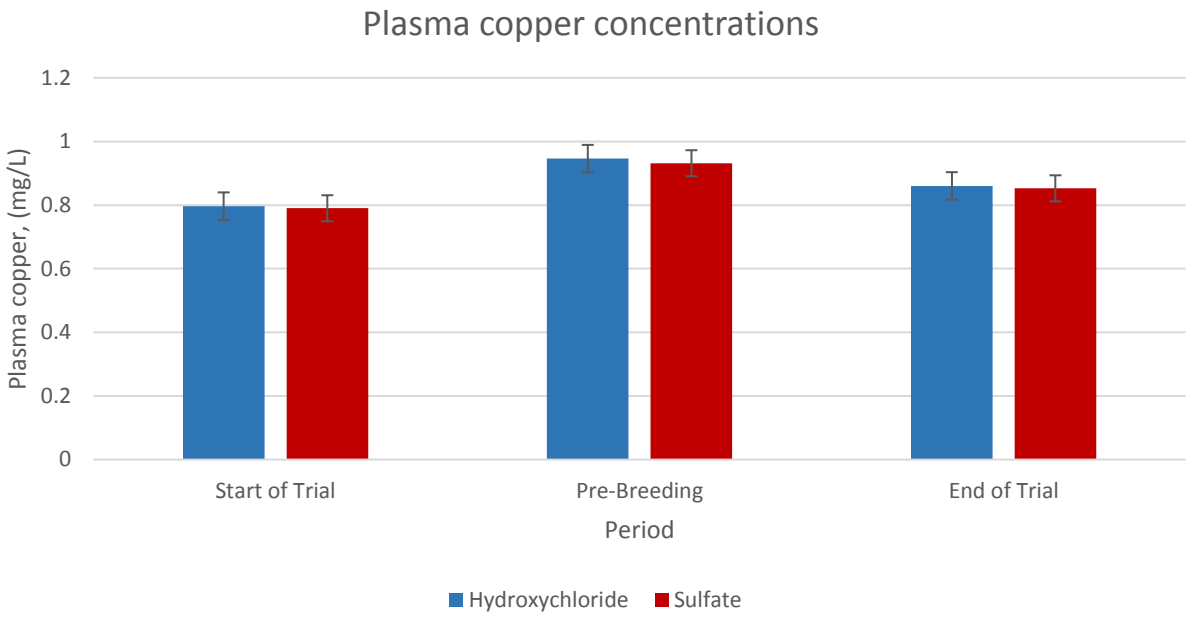

Figure 7. Plasma copper concentration, mg/L. Treatment (P = 0.77); Period (P < 0.0001); Treatment x Period (P = 0.97)

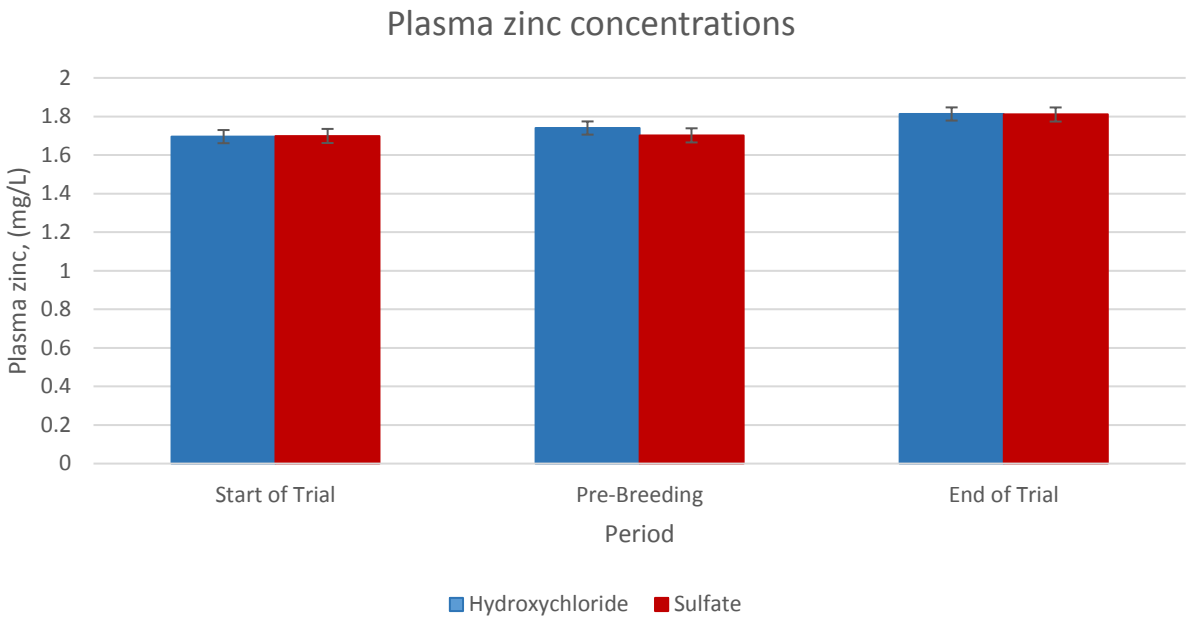

Figure 8. Plasma zinc concentration, mg/L. Treatment ($P = 0.81$); Period ($P < 0.0001$); Treatment x Period ($P = 0.71$)

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UNIVERSITY OF ARKANSAS

Office of Research Compliance

MEMORANDUM

To: Beth Kegley

From: Craig Coon, IACUC ChairDate: June 14, 2015

Subject: IACUC Approval

Expiration Date: July 9, 2018

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your personnel addition of Randy Burnett to protocol # 15066 "Supplemental trace minerals (Zn, Cu, and Mn) as sulfates or hydroxy trace mineral (Zn, Cu, and Mn Intellibond; Micronutrients) sources for beef heifers".

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond July 9, 2018 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem
cc: Animal Welfare Veterinarian

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